CONFORMATIONAL ANALYSIS OF APAMIN USING THE RESIDUAL REPRESENTATION

Bernard BUSETTA
Laboratoire de Cristallographie, LA 144 au CNRS, Université de Bordeaux I, 351, Cours de la Libération, 33405 Talence Cedex, France

Received 27 November 1979
Revised version received 1 February 1980

1. Introduction

Six short non-enzyme peptides were isolated from bee venom. Melittin, the major component and the related melittin F are rather hydrophobic peptides with haemolytic activity. The secapin and tertiapin peptides do not show any activity. The other two, apamin and peptide 401, are basic peptides and seem somehow related. The same location of the disulfide bridges may suggest similar conformations [1] (fig.1), however they show quite different activities.

Apamin is a neurotoxic polypeptide, small enough to pass the blood–brain barrier, which acts specifically at the level of the synaptic mechanisms in the spinal cord [2]. Peptide 401 degranulates mast cells and this entails the release of histamine and 5-hydroxytryptamine and should, therefore, produce inflammation. But, nevertheless, this polypeptide also shows a very strong anti-inflammatory activity. It is not yet established whether these two apparently opposite activities, inflammatory and anti-inflammatory are connected [3].

This is a theoretical investigation on the possible tertiary structures of these two peptides.

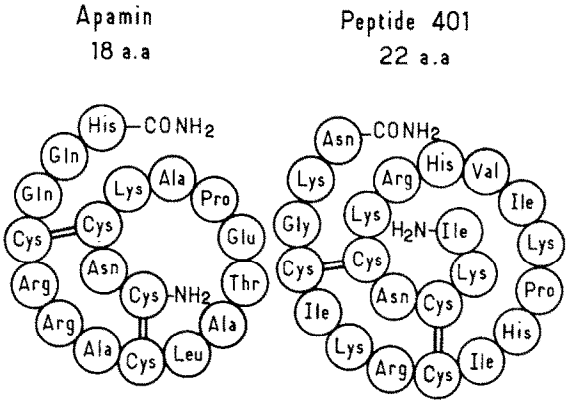

Fig.1. The amino acid sequence of apamin and peptide 401.

2. Materials and methods

In the folded conformation, the position of every atom depends uniquely on the particular amino acid sequence [4]. Protein folding is a physical process depending on the same interatomic forces that stabilize the simplest molecules. In principle, one should be able to use the methods of conformational analysis developed for small molecules on proteins. In practice, severe problems arise when extending techniques that work well for small systems to much larger systems:

(i) Proteins have too many atoms. Calculating the total energy is time consuming.

(ii) Proteins are stable in water at room temperature. The properties of small molecules are computed in vacuo at low temperature. Much less is known about the effect of the solvent and atomic thermal motion on the interatomic forces.

(iii) The presence of too many variables make energy minimization much less efficient. The use of the conventional all atom representation for such problems has been rather disappointing till now [5].

For all these reasons, Levitt's simplified residual representation [6,7] which deals with residue instead of atom is the most suitable one to describe protein folding. The basis of this representation is to average over the finer details and to consider only those degrees of freedom that have the greatest effect on conformation. The results for the folding of bovine

pancreatic trypsin inhibitor [7] and carp myogen [8] are quite satisfactory.

In our first attempts to fold apamin we simultaneously used for Van der Waals parameters, the sets B (side chain only) and C (whole residue) proposed [7]. The first folded conformations of apamin are too compact and without physical meanings. Slight variations of Levitt's representation were necessary.

The main chain cannot have no thickness as in the model with 1 sphere/residue [7]. A 2 sphere model is better:

- One sphere to mime the main chain, centered on the C_α carbon with a radius equal to 4.2 Å;
- One sphere to mime the sidechain, centered as in Levitt's model on the sidechain centroid. With this model only the set B of Van der Waal's parameters is used.

The folding performed for apamin with this new model remained unsatisfactory, and it appeared that the Ala_5 residue wrongly played a prevailing role in the folding process.

Checking the initial model once more showed the implication of the sigmoidal function used to describe the sidechain–solvent interactions, to be the drawback. The approach of an Ala spherical residue by 2 others at the minimum distance produced a loss of surrounding water greater than the initial hydration shell. The computation of the loss of accessible surfaces during the approach of the different spherical residues is better than the use of the empirical sigmoidal function.

All the energy minimizations were performed following the steepest gradient method.

3. Results and discussions

3.1. *The apamin conformation*

In the residual representation the sidechain solvent interactions are the most important factor of the folding process. In an hydrophilic medium the hydrophobic residues would tend to be buried. The folding of apamin from the extended form (all torsion angles = 220°) is performed round the Leu_{10} residue which is the most hydrophobic. The disulfide bridge between Cys_1 and Cys_{11} can be closed in the first step of the folding process. Some rearrangements of the torsion angles of the N-terminal part (Cys–Asn–Cys–Lys) of this first S–S loop are necessary to allow the closing of the second disulfide bridge between Cys_3 and Cys_{15}.

Table 1
The most probable conformations of apamin

	E_{vdw}	$E_{solv.}$	$E_{tors.}$	$E_{tot.}$
Folded conformation (buried Leu_{10})	−13.92	−8.13	−2.54	−24.59
Buried Ala_5	−12.49	−3.33	−5.36	−21.30
Buried Ala_9	−13.25	−1.49	−5.16	−20.08
(1-15, 3-11) S–S bridged apamin				
(a)	−7.68	−6.34	−3.63	−18.22
(b)	−11.26	−2.44	−1.28	−15.04

The continuous folding from the completely extended chain provides the conformation of the lowest energy. Two other conformations (with an Ala_5 or an Ala_9 buried residue) correspond to lower energies but may be in equilibrium with the first more stable conformation

Other different conformations of apamin were built from Dreyding models and refined using the same potential function toward the nearest local energy minimum. These different conformations and their corresponding energies are reported in table 1.

The conformation with Ala_5 or Ala_9 buried are possible but less probable than 'the folded form'. As the energy barriers between these 3 conformations are weak they may coexist in equilibrium even when the S–S bridges are closed. Conversely no stable conformations were obtained with the Ala_{12} or Thr_8 in the buried position. The conformations in which the S–S pairs are interchanged correspond to a higher energy and such a S–S pairing is quite improbable. In fact during the reoxydation of the reduced peptide only the correct pairing occurs [9].

Fig.2a shows the folded chain we got at the end of the folding process of extended apamin. This folded conformation does not display the salt bridge between Glu_7 and the terminal amino group, suggested by NMR spectroscopy [10]. At the beginning of the folding process, short-range interactions are more important than large interactions. This is the reason why we can say that the local burying of the Leu_{10} residue ensures the folding of the polypeptide chain. When the folded conformation is obtained long-range interactions may be established. Fig.2b shows the apamin conformation derived from the folded form if we impose a salt bridge between Glu_7 and the N-terminal amino group. The loss of energy (E_{vdw} = −7.57 kcal, $E_{solv.}$ = −5.75 kcal) may be balanced by the difference of energy involved by the formation of the salt bridge

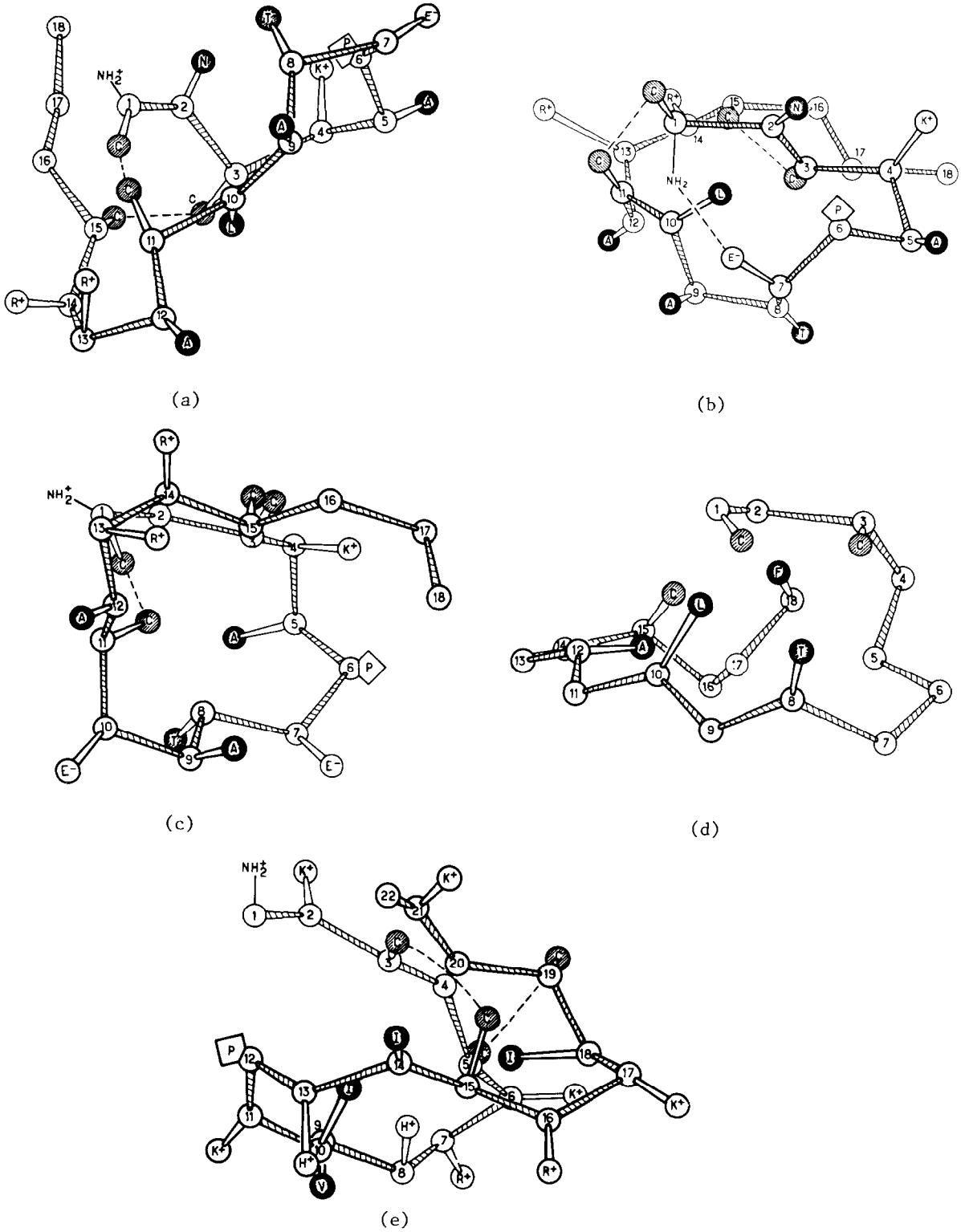

Fig.2. The most probable conformation of apamin analogues and peptide 401. The single letter notation for amino acid (Eur. J. Biochem. (1968) 5, 151–153) is indicated in the corresponding sidechain. Hydrophilic residues are represented by white circles, hydrophobic residues by dark circles. For clarity the three last sidechains are not represented.

that is to say the difference between the energy of a salt bridge (15–20 kcal) and the energy of the polar groups bound to a water molecule (~10 kcal). This last conformation remains different from the most probable conformation of apamin computed using the atomic representation [11] for which a good burying of Leu and Cys residues is not realised.

The conformation of apamin we got shows that both essential positive charges (Arg_{13}, Arg_{14}) are completely exposed to the solvent and pointed nearly in the same direction. However our description is not accurate enough to explain the complete loss of toxicity which occurs when the Arg residues are substituted by Lys [12].

Now we shall show in what manner the residual representation may be used to start the peptide synthesis by an a priori description of the conformation of analogous peptides which would allow the avoidance of undesirable replacements.

What analogues of apamin, having a similar conformation, may be synthesised?

The hydrophilic Lys_4 residue which is not essential for the neurotoxicity [2] is completely exposed. It may be substituted by other strong hydrophilic residues without any change in the folded conformation. The 3 exposed Ala residues could be also substituted by weakly hydrophilic residues. Conversely the Pro residue seems essential to get the 'correct' folded molecule. The Leu_{10} residue must also be essential. Replacing this residue by an hydrophilic one involves a complete change in apamin folding. The folding process of Glu_{10} apamin is easier than that of apamin and the closing of both disulfide bridges is reached without any local minimum. The folded Glu_{10} apamin (fig.2c) looks like the conformation we got for apamin with the Ala_5 buried and should not display any activity. In the same way replacing Ala_5 or Ala_6 by a strongly hydrophobic residue (Leu, Ile, Phe, Tyr) which is able to challenge the Leu_{10}, involves similar conformations.

Three C-terminal residues (Asp–Asn–His) do not seem essential to get the folded conformation and are probably free to move in the surrounding solvent. These 3 residues could be removed without changing the folded conformation too much. But this terminal part of the molecule which is slightly hydrophilic, must not have an hydrophobic character. Substituting His_{18} by a more hydrophobic residue like Phe or Tyr would involve a different folding pathway. The folding process of Phe_{18} apamin leads to a first stable minimum in which the Phe residue is buried in the middle of the folded chain (fig.2d) and it is not easy to close the disulfide bridges. This observation is perhaps related to the loss of toxicity, which decreasing the hydrophilic character of His_8 involves [2].

3.2. *The conformation of peptide 401*

During the folding process of peptide 401 the Ile_{10} and Ile_{18} residues play the same essential role as the Leu_{10} in apamin and are finally quite buried in the folded molecule (fig.2e). Conversely the hydrophobic Ile_{14} residue is pointed towards the solvent on the upper face of the peptide and does not seem essential to get the folded conformation.

If the location of disulfide bridges at the same place in apamin and peptide 401 might suggest similar conformations, the folded molecules appear quite different. Then their quite different activities are not surprising. Peptide 401 is a globular molecule one face of which is covered by 7 basic residues (His_8, His_{13}, Lys_6, Lys_{11}, Lys_{17}, Arg_7, Arg_{16}). Only Lys_2 and Lys_{21} lie on the upper face of the molecule.

4. Conclusions

For small peptides, residual representation may be an important tool in peptide synthesis. It is a cheap way to check whether a peptide analogue will have a suitable conformation before any chemical work. It allowed us to guide the synthesis of active analogues of adrenocorticotropic hormone which will be published later. It is also a good way to study the kinetic rate of disulfide bridge formation.

References

[1] Gauldie, J., Hanson, J. M., Shipolini, R. A. and Vernon, C. A. (1978) Eur. J. Biochem. 83, 405–411.
[2] Vincent, J. P., Schweitz, H. and Lazdunski, M. (1975) Biochemistry 14, 2521–2525.
[3] Banks, B. E. C., Rumjanek, F. D., Sinclair, N. M. and Vernon, C. A. (1976) Bull. Inst. Pasteur 74, 137–144.
[4] Anfinsen, C. B. (1973) Science 181, 223–230.
[5] Burgess, A. W. and Scheraga, H. A. (1975) Proc. Natl. Acad. Sci. USA 72, 1221–1225.

[6] Levitt, M. and Warshel, A. (1975) Nature 253, 694–698.
[7] Levitt, M. (1976) J. Mol. Biol. 104, 59–107.
[8] Warshel, A. and Levitt, M. (1976) J. Mol. Biol. 106, 421–437.
[9] Van Rietschoten, J., Granier, C., Rochat, H., Lissitzky, S. and Miranda, F. (1975) Eur. J. Biochem. 56, 35–40.
[10] Bystrov, V. F., Arseniev, A. S. and Gavrilov, Yu. D. (1978) J. Magn. Res. 30, 151–184.
[11] Popov, E. M. and Mel'Nikov, P. N. (1979) Bioorg. Khim. 5, 1471–1494.
[12] Granier, C., Pedrosomuller, E. and Van Rietschoten, J. (1978) Eur. J. Biochem. 82, 293–298.